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New laboratory criteria of the autoimmune inflammation in pulmonary sarcoidosis and tuberculosis

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ABSTRACT

Sarcoidosis and tuberculosis have many clinical and laboratory similarities, which allowed researchers to assume the presence of common pathogenetic mechanisms in the development of both diseases. Recently, much attention has been paid to investigate the autoimmune origins in these pathologies. The aim of this study is to find out the characteristics of the autoinflammatory immune response in sarcoidosis and tuberculosis. In patients with sarcoidosis ($n = 93$), tuberculosis ($n = 28$), and in healthy donors ($n = 40$), the serum anti-MCV concentration was measured by ELISA, and B cell subpopulations were analyzed by flow cytometry. Based on the results obtained, the formula ([B-naïve%]\[B-memory%]) * ([B-CD38%] + [B-CD5%]) / [anti-MCV] was described. The increase in the calculated index by more than 5 units with a sensitivity of 80.00% and a specificity of 93.10% (AUC = 0.926) suggest the presence of the autoimmune component, which is more typical for sarcoidosis, rather than tuberculosis patients and may serve as a diagnostic criterion.

1. Introduction

Sarcoidosis is a granulomatous disease of unknown etiology characterized with noncaseating granulomas arising in various organs and tissues, most often in the lungs, mediastinal lymph nodes, and skin [1]. Nowadays, more and more scientists consider the autoimmune origin of this disease [2]. Infectious agents including *Mycobacterium tuberculosis, Propionibacterium acnes, Chlamydophila pneumoniae*, viruses (HHV6 and HHV8 herpes viruses, cytomegalovirus, retroviruses), mold [3], as well as environmental triggers are considered to be the most likely etiologic factors. The relationship between sarcoidosis and vaccination, long-term exposure to inorganic dust, dyes, fertilizers, silicon implants as factors triggering autoimmune inflammation has been also described [4–7].

M. tuberculosis appears to play a critical role. Association of sarcoidosis with this infection has been allude with bacterial proteins and their nucleic acids were described in sarcoid granulomas [8,9].

Abbreviations: Anti-MCV, antibodies to mutated citrullinated vimentin; ATS, American Thoracic Society; AUC, area under ROC curve; COPD, chronic obstructive pulmonary disease; ERS, European Respiratory Society; MBT, M. tuberculosis; MSCT, multislice computed tomography; ROC, receiver operating characteristic; SD, sarcoidosis; TB, tuberculosis; Th, T-helpers; WASOG, the World Association of Sarcoidosis and Other Granulomatous Diseases.

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These findings were also supported in studies of the humoral immune response, which showed the presence of antimycobacterial antibodies in the serum of patients [10–12]. The studies of the cellular immune response, according to which lymphocytes with specific cytotoxicity to mycobacteria were found in patients with sarcoidosis [13,14], as well as results of the studies on the animal models of sarcoidosis [15] also support this assumption.

Tuberculosis patients often have autoimmune complications, such as Wegener's granulomatosis, arthritis, and uveitis [16,17]. The possibility of the autoimmune reactions development in tuberculosis has been described in a variety of experimental studies indicating that mycobacteria induced an autospecific T-cell immune response [18–20]. The presence of autoantibodies in tuberculosis patients has been reviewed extensively in recent publications. Thus, high titers of antibodies to cardiolipids, β2-glycoprotein, prothrombin, proteinase-3, neutrophil cytoplasm [21], and cyclic citrulline peptides [22] have been described in patients. Therefore, mycobacteria infection can induce autoimmune processes. Recent research has shown vimentin to be one of the possible autoantigens in sarcoidosis. Wahlström et al. identified vimentin autoantibodies and vimentin-specific T-cells in the bronchoalveolar fluid of sarcoidosis patients [23]. The authors also described the relationship of the HLA-DRB1*03 genotype with the production of these antibodies [21]. Antibodies to vimentin modifications were also detected in tuberculosis patients. According to the study, elevated titers of autoantibodies to modified citrullinated vimentin were found in blood serum of tuberculosis patients [24].

Several researchers have explained the development of autoimmune inflammation in tuberculosis by the presence of the molecular mimicry due to the similarity of immunogenic epitopes of bacteria and autoantigens, resulting in a cross-reaction and activation of T-lymphocytes [25–27]. Despite the existence of possible mycobacterial antigens involved in the cross-reaction (heat shock proteins Mtb-HsP60, Mtb-HsP65, p36 protein, ESAT-6 protein, catalase (mKatG) [28], the bacterial antigen with molecular similarity to vimentin has not been described yet. Perhaps this reaction may be caused by vimentin expressed by macrophages infected with mycobacteria. It has been shown that in the oxidative stress and the presence of pro-inflammatory factors there is an increase in vimentin expression activating the NKp46 receptor of natural killers. This results in the lysis of infected macrophages [29,30].

In addition to the autoantibodies in patients with sarcoidosis, typical changes in B-cell populations are observed [31,32]. An increase in the number of naive B-cells and a decrease in the number of memory B-cells can be detected in granulomas [33,34], blood, and bronchoalveolar fluid [29,30]. Similar changes have been revealed in some autoimmune diseases, such as rheumatoid arthritis and granulomatous polyangiitis [35,36]. The fact that rituximab appeared to be more beneficial in the treatment of diverse clinical forms of the disease is probably one of the confirmations of the active role of B-cells in the pathogenesis of sarcoidosis [37,38].

In tuberculosis, signs of B-cells involvement in the pathogenesis of the disease have also been described [39,40]. According to du Plessis et al., a similar picture characterized by a decreased level of memory B-cells and increased level of naive B-cells have been observed in tuberculosis patients in comparison with healthy subjects. In the treatment of tuberculosis, not only the normalization of the ratio of naive B-cells and memory cells, but also the changing of prevailing memory B-cells population from a switched class to a non-switched class is observed [41].

The aim of the study is to perform a search for the new diagnostic criteria for the presence of autoimmune inflammation in sarcoidosis and pulmonary tuberculosis based on the characteristics of the humoral immune response with an assessment of the level of autoantibodies to modified citrullinated vimentin and the characteristics of the distribution of B-cell subpopulations.

2. Materials of the study

In 2015–2019 years, a prospective comparative study was performed. Patient selection was performed at St. Petersburg Research Institute of Phthisiopulmonology, St.-Petersburg «City Tuberculosis Hospital N№ 2» and St.-Petersburg "City Hospital N№ 2". The study was approved by the independent Ethical Committee of the St. Petersburg Research Institute of Phthisiopulmonology (extract from protocol N№ 34.2 of 01/19/2017) and the Local Ethical Committee of St. Petersburg State University (protocol N№ 01–126 06/30/17). The laboratory part of the study was performed in the Laboratory of autoimmune diseases diagnostics of the First Saint Petersburg State Medical University.

Patients with pulmonary sarcoidosis ($n = 93$), pulmonary tuberculosis ($n = 28$) and healthy subjects ($n = 40$) were enrolled into a study (Table 1). Healthy subjects did not claim any chronic diseases and were not in the contact with tuberculosis patients.

The groups were comparable by gender and age. The exclusion criteria were: a period of more than 2 years after the detection of X-ray changes in the lungs, using immunosuppressive and anti-tuberculosis therapy, plasmapheresis for less than 2 months from the date of inclusion, the presence of HIV infection, syphilis, tumor diseases, decompensated diabetes mellitus.

3. Methods of the study

All patients underwent a complex examination, including clinical assessment, computed tomography (CT) of the chest, laboratory blood tests, a standard set of tests for tuberculosis, histological verification of the lungs lesions and intrathoracic lymph nodes (transbronchial and thoracoscopic biopsy).

The diagnosis of pulmonary sarcoidosis was performed according to the criteria of the American Thoracic Society (ATS), European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Diseases (WASOG). Criteria included typical radiological changes (mediastinal lymphadenopathy, disseminated foci in lung tissue); histological verification of lung lesions or intrathoracic lymph nodes (detection of granulomas of epithelioid cells without caseous necrosis and acid-resistant mycobacteria); the exclusion of other causes of granulomatous changes, especially tuberculosis.

The diagnosis of pulmonary tuberculosis was established using typical radiological changes (mediastinal lymphadenopathy, focal and infiltrative changes with or without destruction); positive results of tuberculosis tests (detection of *M. tuberculosis* (MBT) and/or MTB DNA in sputum with molecular, genetic and bacteriological methods).

The study was approved by the independent ethical committee of the St. Petersburg Scientific Research Institute of Phthisiopulmonology

Table 1
The clinical characteristics of patients with pulmonary sarcoidosis and tuberculosis.

N (%)	Sarcoidosis (n = 93)	Tuberculosis (n = 28)
Men	40 (43.9%)	10 (57.5%)
Women	53 (56.1%)	18 (42.5%)
X-ray examination		
Focal infiltrates in the lungs	93 (100.0%)	25 (100.0%)
Mediastinal lymphadenopathy	76 (81.7%)	11 (39.3%)
Complaints		
Cough	36 (38.7%)	10 (35.7%)
Shortness of breath	24 (25.8%)	12 (42.8%)
Hyperthermia	35 (37.7%)	15 (53.6%)
Weight loss	21 (22.6%)	15 (53.6%)
Chest pain	7 (7.5%)	8 (28.5%)
Immunologic test ELISPOT		
Positive	9 (9.8%)	24 (85.7%)
Negative	84 (90.2%)	4 (14.3%)
Sputum + biopsy specimen microscopy for MBT (positive)	0	28 (100.0)

(extract from protocol No. 34.2 of 01/19/2017) and the Local Ethics Committee of St. Petersburg State University (protocol No. 01–126 06/30/17).

3.1. The antibodies level studies

In the serum of patients with sarcoidosis ($n = 93$), tuberculosis ($n = 28$), and the control group ($n = 40$), the level of antibodies to the modified citrullinated vimentin (anti-MCV) was determined using ELISA (ORGENTEC, Germany). All measurements were performed using a BIOTEK ELx800 ELISA spectrophotometer. A positive result associated with the detection of these antibodies level was considered to be more than 19.5 U/ml.

3.2. Immunotyping

The preparation of peripheral blood samples and tuning of the flow cytofluorimeter was performed by the technique described by S. Khaidukov et al. [42]. The selection of optimal antibody-fluorochrome pairs was performed in accordance with the principles described elsewhere [43]. To identify main B-cell subsets, 200 μl of whole EDTA-stabilized peripheral blood was stained for surface antigens using the following combination of monoclonal antibodies conjugated with fluorochromes: Anti-IgD Alexa Fluor 488 (clone IA6-2, isotype - Mouse IgG2a, κ), anti-CD38 PE (clone LS198-4-3, isotype Mouse IgG1), anti-CD27 PC7 (clone 1A4CD27, isotype Mouse IgG1), anti-CD24 APC (clone J3-119, isotype Mouse IgG1), anti-CD19 APC / Cy7 (clone HIB19, isotype Mouse IgG1, κ), anti-CD5 Pacific Blue (clone BL1a, isotype Mouse IgG2a) and anti-CD45 Krome Orange (clone J33, isotype Mouse IgG1). IgD and CD19 were from BioLegend, Inc. (USA). Antibodies to CD38, CD27, CD24, CD5, and CD45 were from Beckman Coulter, Inc. (USA).

After incubation with antibodies at room temperature in the dark for 15 min, red blood cells were lysed with 2 ml of VersaLyse Lysing Solution (Beckman Coulter, Inc., USA) supplemented with 50 μl of IOTest 3 Fixative Solution (Beckman Coulter, Inc., USA) for 15 min. Next, the samples were washed twice (7 min at 330 g) with phosphate-buffered saline (PBS) supplied with 2% heat inactivated fetal bovine serum (Sigma-Aldrich, USA), the resulting cell pellet was resuspended in 0.5 ml of fresh PBS containing 2% neutral buffered formalin solution (Sigma-Aldrich, USA). Samples were analyzed using a Navios flow cytometer (Beckman Coulter, Inc., USA) equipped with 405, 488 and 638 nm lasers. For each of the samples, at least 10,000 CD19+ lymphocytes were analyzed. Gating strategy was described previously [42]. The data obtained were analyzed using the Kaluza software (Beckman Coulter, Inc., USA). Finally, memory B-cells (IgD–CD27+), naive (IgD + CD27–) and B-regulatory cells (CD24+++CD38+++ and CD5 + CD27–) were identified.

3.3. Statistical processing of results

Statistical analysis was performed using GraphPad Prism 6 (Graph Pad Software, USA), Statistica 10 (Statsoft, USA) using Mann-Whitney criterion, Spearman statistical analysis. ROC analysis was used to determine the diagnostic significance of the results. The differences were considered statistically significant at a p level of less than 0.05, diagnostically significant at AUC > 0.80.

4. Results

4.1. Testing the level of antibodies to modified citrullinated vimentin

The level of anti-MCV was analyzed in 93 patients with sarcoidosis, 28 patients with tuberculosis and 40 healthy subjects, the results are presented in Table 2.

According to the data described in Table 2, an increased anti-MCV level was found in 40.9% (38/93) of sarcoidosis patients and in 60.7% (17/28) of tuberculosis patients. The statistically significant difference was found between the two groups (Mann-Whitney criterion, $p = 0.027$). A high anti-MCV concentration was also determined in 7.5% (3/40) of cases in the group of healthy subjects. Mann-Whitney analysis revealed a statistically significant difference between the sarcoidosis and tuberculosis groups in comparison with the control group ($p < 0.0001$ for both groups) (Fig. 1).

Table 2
The results of the anti-MCV level testing in the study groups.

Study Groups	Results of anti-MCV testing		CI 95%
	An increased level n/%	Absolute value Med (Q25; Q75)	
Sarcoidosis, n = 93	42.2 (38/93)	16.60 (10.73;24.19)	17.0–23.89
Tuberculosis, n = 28	60.7 (17/28)	21.13* (16.50;26.96)	19.17–27.61
Healthy subjects (control group), $n = 40$	7.5 (3/40)	6.47 (2.66;11.25)	5.82–10.65

In order to identify new criteria for determining the level of anti-MCV, ROC analysis was performed. According to the ROC analysis, reference values of the anti-MCV level (AUC > 0.8, p < 0.0001) can be determined for patients with sarcoidosis and tuberculosis in comparison with the control group: 12 units/ml with a diagnostic sensitivity of 77%, specificity of 70% for patients with sarcoidosis, 14 units/ml with a diagnostic sensitivity of 92%, specificity of 88% for patients with tuberculosis. However, no diagnostically significant difference between the two groups was described (AUC <0.8).

4.2. Immunophenotyping tests

An analysis of B-cell populations in the study groups was performed. The most significant B-cell subsets were revealed in comparison with the healthy control group. According to the classification of B-cells by the presence of IgD and CD27, B-cells were divided into “naive” and memory B-cells.

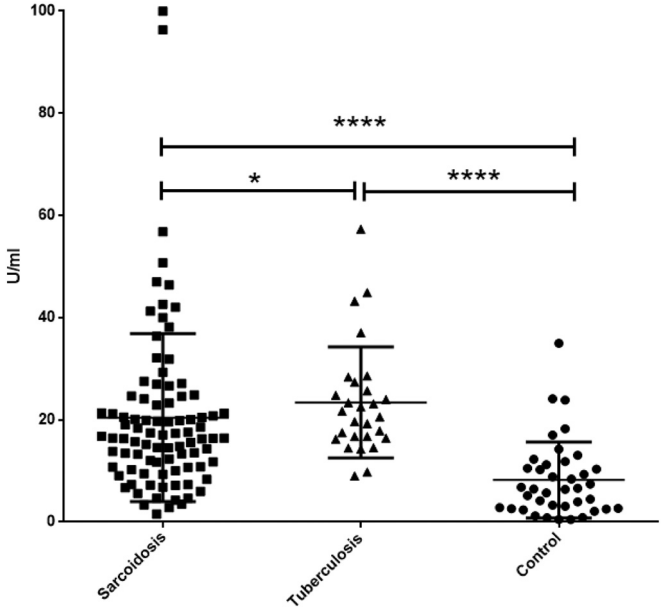

Fig. 1. Distribution of Anti-MCV concentrations in study groups.
* - $p < 0.05$ - comparison between sarcoidosis and tuberculosis groups.
**** - p < 0.0001 - comparison between sarcoidosis and the control group, between tuberculosis and the control group.

4.2.1. "Naive" IgD ± CD27– B-cells

It has been shown that in patients with sarcoidosis and tuberculosis, the number of "naive" B-cells were higher than in healthy donors. The results of "naive" B-cells measurement are presented in Fig. 2.

According to the Mann-Whitney test, the level of "naïve" B-cells in sarcoidosis patients is significantly higher as compared with healthy donors ($p < 0.0001$) and tuberculosis patients ($p = 0.015$). The level of "naïve" B-cells in tuberculosis patients is also significantly higher than the number of "naïve" B-cells in control group ($p = 0.007$) (Fig. 2).

According to the ROC analysis results, in comparison with the control group, diagnostically significant results were obtained for sarcoidosis patients alone (AUC = 0.819, $p < 0.0001$). For this group, the reference value for "naïve" B-cells number was 70% with diagnostic sensitivity of 76%, specificity - 70%. There was no diagnostically significant difference between the sarcoidosis and tuberculosis groups.

4.2.2. IgD–CD27+ memory B-cells

The level of memory B-cells in patients with sarcoidosis and tuberculosis appeared to be lower than in the control group. The results of memory B-cells analysis are presented in Fig. 3.

According to the Mann-Whitney test, the levels of memory B-cells in sarcoidosis and tuberculosis groups were significantly lower than in healthy controls ($p < 0.0001$ and $p = 0.007$, respectively). Furthermore, the significant difference was also found between sarcoidosis and tuberculosis groups ($p = 0.005$).

Using the ROC analysis, diagnostically significant results were determined only for sarcoidosis patients as compared with the control group (AUC = 0.819). In patients with sarcoidosis, the level of memory B-cells was below 30% with a diagnostic sensitivity of 76% and a specificity of 70%. There was evidence of significant difference between sarcoidosis and tuberculosis groups. By labeling the antigens CD24, CD38, CD5, CD27, group B of regulatory cells was determined, in which

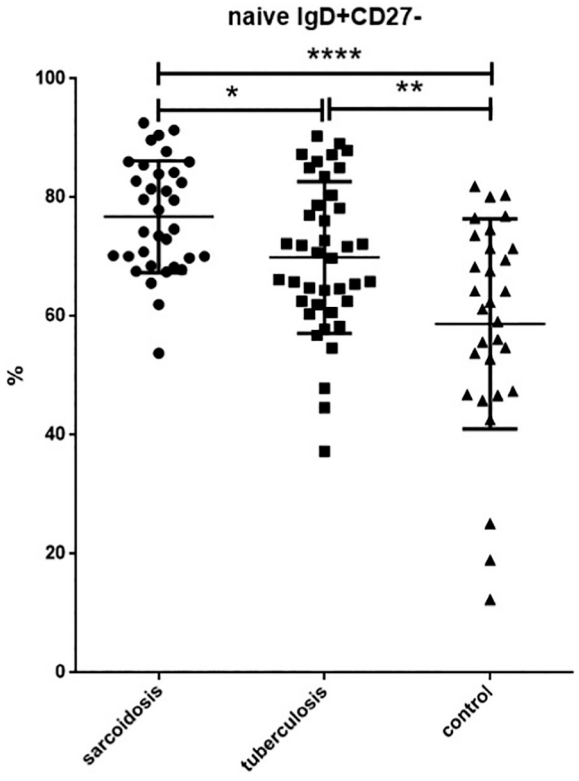

Fig. 2. Distribution of "naive" IgD + CD27– B-cells level among study groups.
* - $p < 0.05$: between sarcoidosis and tuberculosis.
** - $p < 0.01$: between tuberculosis and healthy subjects.
**** - $p < 0.0001$: between sarcoidosis and healthy subjects.

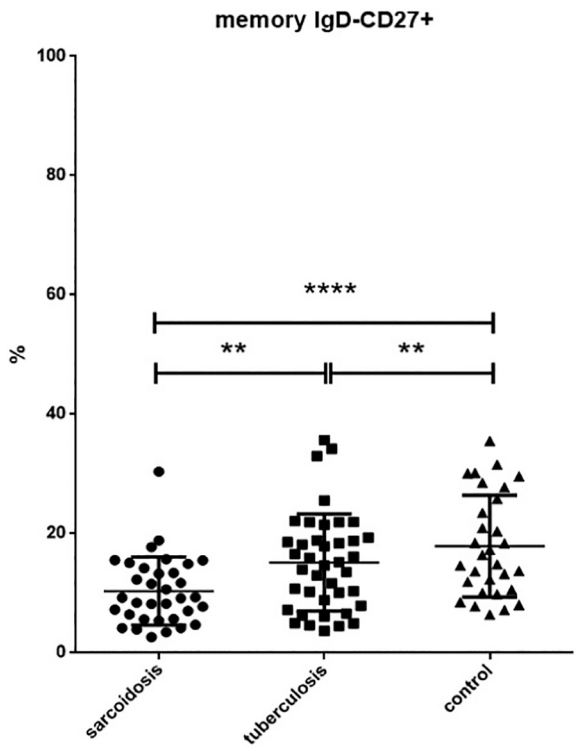

Fig. 3. Distribution of the level of IgD–CD27 + memory B-cells among study groups.
** - $p < 0.01$: between sarcoidosis and tuberculosis, between tuberculosis and control.
**** - $p < 0.0001$: between sarcoidosis and control.

CD24+++CD38+++ B cells and CD5 + CD27– cells were isolated.

4.2.3. CD24+++CD38+++ B-cells

The level of CD24 +++ CD38 +++ B-cells was elevated in patients with sarcoidosis and tuberculosis in comparison with the control group. The results of measuring the level of CD24 +++ CD38 +++ B-cells are presented in Fig. 4.

The Mann-Whitney test showed a significant increase in CD24+++CD38+++ B cells patients with sarcoidosis and tuberculosis as compared with healthy donors ($p < 0.0001$ in both cases). There was no significant difference between patient groups with sarcoidosis and tuberculosis.

According to the ROC analysis, a diagnostically significant increase in CD24 +++ CD38 +++ B cells regarding healthy individuals was found only in patients with sarcoidosis (AUC = 0.904), the reference value was 6.52% with a diagnostic sensitivity of 91%, specificity of 88%. There was no significant difference between the groups of sarcoidosis and tuberculosis.

4.2.4. CD5 ± CD27- B cells

An increase in CD5 + CD27- B-cells levels of regarding healthy individuals was found in sarcoidosis and tuberculosis groups. The results of measuring the level of CD5 + CD27-B-cells are presented in Fig. 5.

According to the Mann-Whitney test, the level of CD5 + CD27- B-cells appeared to be higher in sarcoidosis patients as compared with healthy donors ($p < 0.0001$) and tuberculosis patients ($p = 0.001$).

The ROC analysis revealed a diagnostically significant increase in CD5 + CD27–B -cells regarding the control group of more than 12.45% for sarcoidosis patients (AUC = 0.795) with a diagnostic sensitivity of 76%, specificity of 80%. There was no evidence of diagnostically significant difference between the sarcoidosis and tuberculosis groups.

Thus, according to the ROC analysis of a significant difference in the

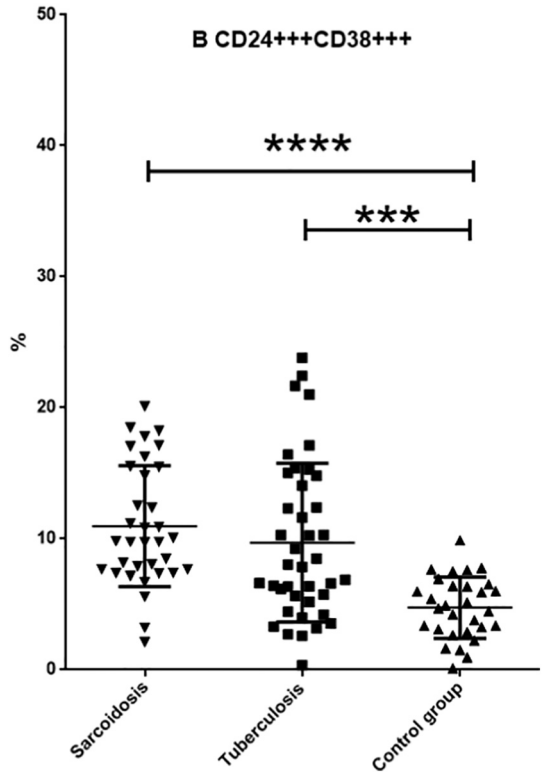

Fig. 4. Distribution of the level of CD24 +++ CD38 +++ B-cells among study groups.
*** - $p < 0.005$: between tuberculosis and control group.
**** - $p < 0.0001$: between sarcoidosis and control group.

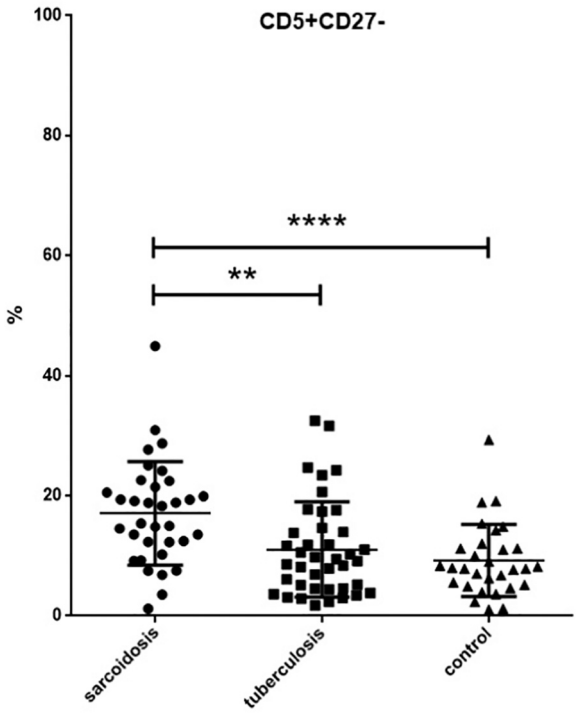

Fig. 5. Distribution of the level of CD5 + CD27– B-cells among study groups.
** - $p < 0.01$: between sarcoidosis and tuberculosis.
**** - p < 0.0001: between sarcoidosis and control group.

level of anti-MCV, the number of "naive" IgD + CD27-B-cells, IgD-CD27 + memory B-cells, CD24 +++ CD38 +++ B-cells, CD5 + CD27-B-cells was not found. A comprehensive analysis of the data was performed in order to establish a diagnostic index.

The search for a comparative index has shown that when using the formula (1) with a sensitivity of 80.00% and specificity of 93.10% the obtained index of more than 5 units (from 3.8 and more for sarcoidosis; 0.2–18 for tuberculosis) indicates a high probability of the presence of sarcoidosis (AUC = 0.926).

$$Ds = \frac{Bnaive}{Bmemory} * \frac{(CD38 - Bcells) + (CD5 - Bcells)}{[anti - MCV]} \quad (1)$$

(B-naive \ B-memory) is the index of the humoral immune response activity;

(CD38-B-cellls + CD5-B-cells) - the total number of regulatory B cells (Il-10 synthesis);

[anti-MCV] - the concentration of anti-MCV, characterizing the presence of autoimmune reactions.

5. Discussion

An increased level of autoantibodies to citrullinated modified vimentin is descrined both in sarcoidosis and tuberculosis patients ($p < 0.0001$) suggesting the presence of autoimmune inflammation in both diseases.

The presence of high concentrations of autoantibodies to MCV may be explained by the high immunogenicity of this antigen. Vimentin is a protein of connective tissue and can be found in many cells. The development of autoimmune processes against citrullinated vimentin is described in rheumatoid arthritis, systemic lupus erythematosus, and other autoimmune diseases [44]. The processes of citrullination are known to be necessary for the arginine-containing proteins elimination (vimentin, fibrin, and others) by macrophages [45,46]. However, in chronic inflammation, permanent tissue damage occurs, which results in an increase in calcium concentration and hyperactivation of peptidyl-arginine deaminase, an enzyme involved in protein citrullination [47]. For the carriers of the HLA-DRB1 genotypes, this process contributes to the activation of autoimmune reactions against citrullinated peptides [48]. A similar process has been described in the pathogenesis of rheumatoid arthritis and chronic pulmonary diseases, in which high titers of antibodies to citrulline proteins are found in patients [45].

According to our previous work, high anti-MCV titers were not detected in alveolitis and polyangiitis [24]. Taking into account that there was no diagnostically significant difference in the anti-MCV levels in both sarcoidosis and tuberculosis, and that the reference level of anti-MCV for both groups was less than the recommended value for the diagnosis of rheumatoid arthritis, it can be suggested that in tuberculosis infection an autoimmune inflammation against vimentin antigen could exist, which, under certain conditions, can become systemic and result in the formation of sarcoid granulomas. Determination of anti-MCV levels over 12 units/ml may allude granulomatous inflammation in the lungs, but, the testing of this biomarker alone can't be applied for the differentiation diagnosis between tuberculosis and sarcoidosis.

Immunophenotyping analysis has shown that patients with tuberculosis and sarcoidosis had reduced level of memory B-cells, an increased number of "naïve" B-cells and CD24 +++ CD38 +++ B-cells in comparison with healthy controls. Moreover, in sarcoidosis patients the memory B-cells level was considerably lower while the level of "naive" B-cells was higher than in tuberculosis patients.

In sarcoidosis patients, the level of CD5 + CD27– B cells was statistically higher than in blood samples from both healthy individuals and tuberculosis patients.

Alterations in B-cell subsets may be closely related to the inflammatory reactions. This is an essential part of foreign antigen elimination. However, it remains unclear what cells characteristics may indicate the presence of an autoimmune process.

According to our data, in sarcoidosis and tuberculosis patients, there was an increase in "naive" B-cells and a reduction in memory B-cells numbers, though, the imbalance of memory B-cells and "naive" B-cells in sarcoidosis patients was more pronounced, and it was confirmed by statistical analysis. Similar results were obtained in the study of chronic sarcoidosis [32] and active forms of tuberculosis [41]. According to the data of du Plessis et al., the described changes in B-cell subsets were found only in tuberculosis patients in comparison with other lung diseases (viral or bacterial pneumonia, bronchiectasis, asthma, COPD), which did not statistically differ from the group of healthy individuals [41]. According to O'Shea et al., during active tuberculosis, a decrease in memory B-cells was detected, while the frequency of "naive" B-cells was unaltered [49]. Impaired memory and "naïve" B-cells distribution has been described in some autoimmune diseases [35,36].

Taking into account the absence of changes in the ratio of memory and "naive" B-cells in other pulmonary diseases, it can be postulated that such changes may develop in diseases in which granulomatous inflammation plays an important role. This is typical of some infectious and autoimmune diseases.

Analysis of CD24+++CD38+++ B-cells has revealed their increased levels in patients with sarcoidosis or tuberculosis versus healthy controls, though there was no significant difference between those two patient groups. It is known that CD24+++CD38+++ B-cells represent a population of immature transitional B-cells that perform various regulatory functions [50]. One of their main characteristics is the ability to synthesize the anti-inflammatory cytokine IL-10 [51]. Many studies have shown that B-cells producing IL-10 could inhibit the activity of self-specific CD4 + T-cells [52]. A decrease in CD24+++CD38+++ B-cell levels was found in patients with rheumatoid arthritis, which allowed investigators to confirm the role of CD24+++CD38+++ B-cells in preventing autoimmune reactions [53]. However, an increase in the level of these cells was noted in primary Sjogren's syndrome and systemic lupus erythematosus [54], as well as in patients with an active phase of sarcoidosis [31]. According to Blair, in patients with systemic lupus erythematosus there were lower levels of $CD38^{int}CD24^{int}$ and CD24+++CD38–B-cells while the level of CD24+++CD38+++ − cells was increased [51]. Probably, the distribution disorder of those B-cells indicates the activation of a compensatory anti-inflammatory response in infectious and autoimmune diseases. In autoimmune processes an activation of immune system might be replaced by decompensation, characterized by a decrease in the level of these cells.

There is no evidence of data explaining CD24+++CD38–B-cells alterations during inflammatory processes in infectious diseases.

Interesting results were obtained when comparing CD5-expressing B-cells. CD5-expressing B-cells also refer to regulatory B-cells capable of synthesizing IL-10. They can be found in a variety of human tissues. Moreover, CD5+ B-cells are capable of producing autoantibodies (including rheumatoid factor and antibodies against ssDNA), and the number of CD5+ B-cells increases in autoimmune diseases such as rheumatoid arthritis and Sjogren's syndrome [55,56]. The ability of cells to produce autoantibodies was revealed in mouse model. It was shown that CD5-expressing B-cells belonging to a subpopulation of B1a, which is usually localized in the abdominal cavity, produce low-affinity IgM antibodies with autoreactive specificity [57]. In addition, IL-10 synthesized by CD5+ B-cells takes part in the control of autoimmune reactions in the experimental encephalomyelitis in mice [58]. In humans, CD5 is found on the cell membrane of transient CD24+++CD38++ T1 B-cells [59], but according to recent studies, these cells produce a relatively low level of IL-10 as compared to other transient B-cell subsets [54]. There is evidence that CD5 can be considered as a marker of activation of B-cells in humans, and CD5 negative human B-cells can be activated in vitro by incubation with phorbol or thymoma EL4 cells, followed by the appearance of CD5 molecules on their membrane [60]. According to Zhang et al., elevated levels of CD5-expressing B-cells can also be detected in tuberculosis patients. At the same time, the ability of these cells to inhibit Th17 activity has been shown [61].

Our study has revealed a statistically significant increase in the number of B-cells with the CD5 + CD27– phenotype only in sarcoidosis patients. Currently, it is difficult to explain the absence of differences in the levels of CD5 + CD27– B-cells in patients with tuberculosis and healthy individuals, perhaps the results obtained are related to a small sample size.

According to the ROC analysis of immunophenotyping results, there were no diagnostically significant findings for the differentiation of sarcoidosis and tuberculosis, however, a tendency to more pronounced changes in the level of B-cells in patients with sarcoidosis is evident.

Our study showed the presence of autoimmune processes in sarcoidosis and tuberculosis, namely, an increase in anti-MCV more than 14 U/ml, an increase in "naive" B-cells and a decrease in memory B-cells, an increase in CD24 ++ + CD38 +++ B-cells. The mathematical formula was developed according to which, with an increase in the index of more than 5 units, the presence of sarcoidosis is more likely, which may reflect a more significant autoimmune inflammation in sarcoidosis compared with tuberculosis.

6. Conclusion

Sarcoidosis and tuberculosis have similar immunologic changes considering the possible presence of autoimmune inflammation and are characterized by an increase in anti-MCV more than 14 U/ml, an increase in "naive" B-cells and a decrease in memory B-cells, an increase in CD24 +++ CD38 +++ B- cells.

Application of the formula with a sensitivity of 80.0% and a specificity of 93.10% suggests the presence of sarcoidosis with an increase in the calculated index by more than 5 units (AUC = 0.926). The resulting indicator may show that changes in B-cell subpopulations has autoimmune origin and are specific to sarcoidosis.

To confirm the diagnostic significance of the described criteria, it is necessary to perform studies on the application of the described formula, determining an increase in the level of anti-MCV more than 14 U ml, a change in the ratio of memory and "naive" B cells, an increase in the number of CD24 +++ CD38 +++ B cells in groups of patients with various autoimmune and granulomatous diseases, which will make it possible to determine more accurate and specific values for the differential diagnosis of sarcoidosis and tuberculosis, that has great practical application.

Ethics approval and consent to participate

The study was approved by the independent ethical committee of the St. Petersburg Scientific Research Institute of Phthisiopulmonology (extract from protocol No. 34.2 of 01/19/2017) and the Local Ethics Committee of St. Petersburg State University (protocol No. 01–126 06/30/17).

Consent for publication

"Not applicable"

Availability of data and material

The data will not be shared with a reason.

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Declaration of Competing Interest

"The authors declare that they have no competing interests."

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